A Simple Soil Test for Detecting Sites that are Nonresponsive to Nitrogen Fertilization

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ABSTRACT

Recent work indicates that accumulation of amino sugar N in soil reduces the yield response of corn (*Zea mays* L.) to N fertilization, and that nonresponsive sites are detectable by determination of amino sugar N in soil hydrolysates. Unfortunately, the hydrolysis process is too complicated and time-consuming for use in routine soil testing. A much simpler technique was developed to estimate amino sugar N without the need for acid hydrolysis. In this test, 1 g of air-dried soil is treated with 10 mL of 2 *M* NaOH in a 473-mL (1-pint) wide-mouth Mason jar, and the sample is heated for 5 h at 48 to 50°C on a hot plate to liberate (NH_4 + amino sugar)-N as gaseous NH_3. The NH_3 is collected in H_3BO_3–indicator solution, and subsequently determined by acidimetric titration. Recovery ranged from 97 to 102% when analyses were performed after treating samples with ^{15}N-labeled $(NH_4)_2SO_4$ or glucosamine, but did not exceed 6.5% with labeled glycine and was undetectable with labeled NO_3 or NO_2. Comparative studies using 12 nonresponsive and 13 responsive soils showed a very high correlation between soil-test N and hydrolyzable amino sugar N (r = 0.90*). Test values were significantly higher ($P < 0.001$) for nonresponsive (237–435 mg N kg^{-1}) than for responsive (72–223 mg N kg^{-1}) soils. The soil test described has important economic implications for production agriculture, and also should be of value for controlling NO_3 pollution of ground and surface water.**

Soil testing is used routinely to guide agricultural applications of limestone, P, and K, whereas N applications for corn production are often based on a yield goal, with adjustments to allow for other N inputs, such as legumes and manure. A yield-based recommendation may have merit on a long-term basis, but under or overfertilization is apt to occur in any given growing season since soil N availability is not taken into account. Insufficient application of N can have serious economic consequences for the farmer, whereas excessive fertilization increases the risk of environmental pollution.

Estimation of plant-available N is complicated enormously by the dynamic nature of soil N, owing largely to the effects of temperature and moisture supply on N-cycle processes. Numerous biological and chemical methods have been proposed as an index of soil N availability (Bremner, 1965; Keeney, 1982; Stanford, 1982; Bundy and Meisinger, 1994), but none has been adopted widely for soil testing. Biological methods are necessarily time-consuming because of the need for incubation, and the results represent the net effect of mineralization-immobilization turnover rather than gross mineralization. Chemical methods of estimating potentially mineralizable soil N have been based on an empirical approach, and their use has been very limited because of low correlations with the production of mineral N and crop N uptake.

Soil testing for NO_3 is currently considered the best option for identifying sites where N fertilization will be ineffective in producing a yield response by corn (Bundy and Meisinger, 1994). Two soil NO_3 tests have been developed that differ in the time and depth of sampling. With the preplant NO_3 test (PPNT), profile samples are collected in the early spring to a depth of 60 or 90 cm, to account for carryover of mineral N from previous cropping (e.g., Bundy and Malone, 1988; Roth and Fox, 1990; Schmitt and Randall, 1994). With the presidedress NO_3 test (PSNT), soil sampling is done to a depth of 30 cm in late spring, so that soil N mineralization can be taken into account and supplemented, if necessary, by sidedressing (e.g., Magdoff et al., 1984; Fox et al., 1989; Blackmer et al., 1989; Meisinger et al., 1992; Bundy and Andraski, 1993). The PSNT has been recommended more widely than the PPNT in the eastern USA, but usage has been limited by the need to collect soil samples during the growing season, and by the fact that N fertilization must be postponed until after testing and can be ineffective if adverse weather conditions delay sidedressing. Besides logistical problems, an inherent limitation with the PPNT and PSNT arises from the extensive spatial and temporal variability in soil NO_3 concentrations, which depend on numerous N-cycle processes, including mineralization, immobilization, nitrification, denitrification, leaching, and plant uptake. Consequently, a one-time test for soil NO_3 is apt to be of little value for predicting crop N availability throughout the growing season, particularly in a humid region where these processes occur extensively.

Ideally, a soil test for N would estimate a labile organic fraction that supplies the plant through mineralization. Such an approach would have the major advantage over NO_3 testing that soil test levels would depend on fewer N-cycle processes and should, therefore, be less variable. This would make the time of soil sampling much less critical than with NO_3 testing, so that soil N availability could potentially be predicted on the basis of a one-time test prior to the growing season.

Numerous attempts have been made to identify a labile pool of soil organic N through chemical fractionation of the N in soil hydrolysates (e.g., Keeney and Bremner, 1964; Porter et al., 1964; Khan, 1971; Smith and Young, 1975; Meints and Peterson, 1977), but with little tangible progress. The stagnation can be attributed, at least in part, to serious defects in methodology that vitiated analyses for amino sugar N and amino acid N. These defects were identified and eliminated through a substantial effort that ultimately led to simple diffusion

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Abbreviations: LSD, least significant difference; PPNT, preplant nitrate test; PSNT, presidedress nitrate test; *** significant at the 0.001 probability level.

methods of fractionating the N in soil hydrolysates (Mulvaney and Khan, 2001).

In several recent studies throughout the north-central and northeastern USA, numerous sites have been detected where corn does not respond to N fertilization (e.g., Bundy and Malone, 1988; Blackmer et al., 1989; Fox et al., 1989; Roth and Fox, 1990; Meisinger et al., 1992; Brown et al., 1993; Schmitt and Randall, 1994). Such sites are often associated with recent manuring or the presence of a previous forage legume, but this was not the case with many of the 33 nonresponsive sites detected in an Illinois study that involved 75 site–years (Brown et al., 1993). The newly developed diffusion methods were applied to soil samples collected from responsive and nonresponsive sites in the latter study, so as to ascertain whether a specific fraction of soil organic N might be implicated in nonresponsiveness to N fertilization. The results showed a much higher concentration of amino sugar N for nonresponsive than for responsive soils, whereas no consistent difference was detected in their concentrations of total hydrolyzable N, hydrolyzable NH_4-N, or amino acid N (Mulvaney et al., 2001). In subsequent incubation studies reported in the latter publication, nonresponsive soils produced a much larger quantity of mineral N than did responsive soils, and mineralization was accompanied by a net decrease in amino sugar N but not in amino acid N.

The methods employed by Mulvaney et al. (2001) to differentiate between responsive and nonresponsive soils require hydrolysis with 6 *M* HCl for 12 h, followed by filtration and neutralization of the hydrolysate, and are therefore unsuitable for routine soil testing. The purpose of the work reported here was to develop a much simpler technique, whereby amino sugar N can be readily estimated to detect sites that do not require N fertilization. The soil test developed was evaluated relative to analyses of hydrolyzable amino sugar N, and by comparing test values for soils that differed widely in the yield response of corn to N fertilization.

MATERIALS AND METHODS

Soil Samples

Most of the soils used in the present project were PPNT samples (0–30 cm) that had been collected in late March or early April of 1990, 1991, or 1992 from 25 of the 75 sites studied by Brown et al. (1993). At each site, N was subsequently applied at six rates according to a randomized complete block design with four replications, by sidedressing urea-NH_4NO_3 solution (360 g N L^{-1}) when corn was 15 to 30 cm tall. The particular samples used, representing a wide range of textural classes and management practices, were selected from 25 sites receiving normal rainfall, and included samples from 12 sites where no statistically significant ($P < 0.10$) yield response had been observed to N fertilization. In each case, a composite sample of air-dried soil was prepared by combining equal weights of the replicate samples, so as to integrate block effects in comparing different sites. The entire sample was ball-milled to pass through a 0.15-mm screen and was then stored in a Mason jar sealed with an air-tight lid. Additional details concerning the design of the field experiment, plot layout, and soil sampling are provided by Mulvaney et al. (2001).

The 25 sites studied herein are characterized in Table 1, which shows the soil type and physicochemical properties, the previous crop, the tillage system in use, the type and amount of manure applied, PPNT, PSNT, and check-plot corn yield data from Brown (1996), and the percentage yield response by corn to N fertilization at the optimal N rate. Of the chemical analyses reported in Table 1, data for pH, available P, and exchangeable K were obtained from Brown (1996), and organic C and total N were determined as described by Mulvaney and Kurtz (1982). Applications of manure N were estimated on the basis of the quantity of material applied as indicated by farmer records, and the average N concentration according to the Illinois Agronomy Handbook (1998). The percentage yield response by corn to N fertilization was calculated as 100 × (optimum yield − check-plot yield)/check-plot yield, using data reported by Brown (1996) for check-plot yield (i.e., the yield without sidedressing, as reported in Table 1) and optimum yield. The latter value was determined by fitting N rate and corresponding yield data to a quadratic plateau model by nonlinear regression (SAS Institute, 1993).

Profile samples of two nonresponsive and two responsive soils were used in a study to compare soil test values for different sampling depths. These samples were obtained from a depth of 0 to 15, 15 to 30, or 30 to 60 cm.

Four additional Illinois surface (0–15 cm) soils were used for some of the studies reported, including three Mollisols collected from fields under soybean [*Glycine max* (L.) Merr.] production and a Histosol obtained from a permanently waterlogged site. Before use, each sample was air dried and crushed to pass through a 2-mm screen. Their physicochemical properties are reported in Table 2.

Soil Hydrolysates and Hydrolyzable Nitrogen

To prepare soil hydrolysates, 5-g samples of soil (four replicates) were heated (110–120°C) under reflux for 12 h in 125-mL Erlenmeyer flasks fitted with a 24/40 ground-glass joint for attachment to a 40-cm Liebig condenser, after treatment with 20 mL of 6 *M* HCl and two drops of octyl alcohol. The hydrolysis mixture was filtered through Whatman no. 50 filter paper (Whatman, Maidstone, UK) under vacuum. Replicate hydrolysates were combined, and subsequently neutralized by addition of NaOH (Mulvaney et al., 2001). The hydrolysates were stored under refrigeration (5°C).

Using the diffusion methods described by Mulvaney and Khan (2001), the soil hydrolysates were analyzed for total hydrolyzable N, NH_4-N, (NH_4 + amino sugar)-N, and amino acid N. Amino sugar N was determined as the difference between (NH_4 + amino sugar)-N and NH_4-N.

Soil Test Method

Apparati

Diffusion unit. The diffusion unit used consists of a 473-mL (1-pint) wide-mouth Mason jar equipped with a lid that has been modified to support the bottom of a 60-mm (dia.) Pyrex petri dish (Corning Glass Works, Corning, NY). The necessary modifications are described in detail by Mulvaney (1996), Khan et al. (1997), and Mulvaney et al. (1997a). Further information about Mason-jar diffusion methodology can be found therein, including a description of the appropriate cleaning procedures.

Electric hot plate. A commercial griddle (Model 76212; West Bend, West Bend, WI) was used. Before use, the heat control was adjusted so that a temperature of 48 to 50°C was

Table 1. Characterization of study sites.†

No.	Soil Series‡	Soil Subgroup	pH§	Organic C	Total N	Available P	Available K	Previous crop¶	Tillage#	Manure N applied††	PPNT‡‡	PSNT§§	Yield without sidedressed N¶¶	Fertilizer response##
				g kg^{-1}		kg ha^{-1}				kg ha^{-1}	mg M kg^{-1}		Mg ha^{-1}	%
						Nonresponsive sites								
1	Drummer sicl	fine-silty, mixed, superactive, mesic Typic Endoaquoll	6.6	23.3	2.28	224	896	C_d	M	>1120 (S_l)	27	39	14.0	0.1
2	Maumee ls	sandy, mixed, mesic Typic Endoaquoll	6.3	13.2	1.28	147	406	S	M	500 (S_l)	8	11	13.7	−0.1
3	Flanagan sil	fine, smectitic, mesic Aquic Argiudoll	6.1	21.2	1.99	66	417	A	C	0	7	21	12.8	−0.2
4	Downs sil	fine-silty, mixed, superactive, mesic Mollic Hapludalf	6.5	26.2	2.57	96	269	S	M	190 (P)	23	51	12.3	0.1
5	Drummer sicl	fine-silty, mixed, superactive, mesic Typic Endoaquoll	7.1	27.8	3.49	202	529	C_d	M	2510 (S_l)	20	25	12.1	3
6	Tama sicl	fine-silty, mixed, superactive, mesic Typic Argiudoll	6.1	19.3	2.12	292	788	C	M	85 (BS)	8	11	11.2 (27)	0.1
7	Muscatine sicl	fine-silty, mixed, mesic Aquic Hapludoll	5.7	16.3	1.92	159	470	S	N	210 (S_l)	11	34	10.6 (22)	0.2
8	Cisne sil	fine, smectitic, mesic Vertic Albaqualf	5.2	14.3	1.06	249	260	C	M	0	12	8	10.2 (30)	−0.1
9	Milford sicl	fine, mixed, superactive, mesic Typic Endoaquoll	5.7	20.3	2.26	193	600	S	M	251 (S_l)	38	55	9.3	0.2
10	Flanagan sil	fine, smectitic, mesic Aquic Argiudoll	6.8	20.6	2.13	178	756	S	M	100 (D)	9	17	9.1	0
11	Downs sil	fine-silty, mixed, superactive, mesic Mollic Hapludalf	6.9	14.3	1.61	171	679	C	M	0	10	13	8.8	−0.3
12	Drummer sicl	fine-silty, mixed, superactive, mesic Typic Endoaquoll	6.3	21.1	2.03	81	394	S	N	0	4	7	6.9	0.3
						Responsive sites								
13	Varna sil	fine, illitic, mesic Oxyaquic Argiudoll	6.1	14.6	1.50	71	510	S	N	0	4	5	8.8	46
14	Raddle sil	fine-silty, mixed, superactive, mesic Typic Hapludoll	5.2	11.7	1.16	292	721	S	M	0	5	4	8.8	30
15	Ipava sil	fine, smectitic, mesic Aquic Argiudoll	6.8	20.2	1.79	141	508	C	N	0	3	7	8.7 (34)	17
16	Maumee ls	sandy, mixed, mesic Typic Endoaquoll	6.9	12.2	1.22	119	395	S	M	0	5	8	8.6 (50)	42
17	Cisne sil	fine, smectitic, mesic Vertic Albaqualf	5.5	18.5	1.65	140	565	C	M	0	4	2	7.0	76
18	Atterberry sil	fine-silty, mixed, superactive, mesic Udollic Endoaqualf	6.1	8.8	0.85	73	193	W	M	0	1	6	6.9 (35)	47
19	Iva sil	fine-silty, mixed, mesic Aeric Endoaqualf	5.3	12.8	1.32	23	449	WM	M	0	3	3	6.9 (25)	81
20	Bonfield 1	loamy-skeletal, mixed, mesic Aquic Hapludoll	6.4	18.4	1.84	52	407	S	M	0	2	7	6.7 (7)	68
21	Ipava sil	fine, smectitic, mesic Aquic Argiudoll	5.8	15.9	1.52	94	722	S	N	0	ND	3	6.2	64
22	Varna sil	fine, illitic, mesic Oxyaquic Argiudoll	6.1	20.5	1.87	57	427	S	M	0	4	6	5.6	134
23	Ipava sil	fine, smectitic, mesic Aquic Argiudoll	6.3	14.9	1.51	86	506	S	M	0	4	7	5.6	76
24	Cisne sil	fine, smectitic, mesic Vertic Albaqualf	6.3	9.0	1.09	152	220	C	M	0	4	6	5.0	68
25	Stronghurst sil	fine-silty, mixed, superactive, mesic Aeric Endoaqualf	6.4	5.8	0.61	110	282	WS	N	0	3	3	4.0 (16)	122

† All analytical data are reported as the mean of four replicate determinations.
‡ The suffix included with each series name indicates the textural class of the surface 30 cm: sicl, silty clay loam; ls, loamy sand; sil, silt loam; l, loam.
§ Soil/water ratio, 1:1.
¶ C, corn (*Zea Mays* L.); S, soybean (*Glycine max* L. Merr.); A, alfalfa (*Medicago sativa* L.); W, wheat (*Triticum aestivum* L.); WM, double-cropped wheat and milo (*Sorghum bicolor maili*); WS, double-cropped soybean and wheat. A subscripted d indicates manure disposal during the previous growing season, in lieu of cropping.
# M, mulch; C, conventional; N, no-till.
†† Values indicate the total amount of N applied as manure for the growing season studied, estimated as the product of manure application rate and average N concentration (Illinois Agronomy Handbook, 1998). Abbreviations in parentheses indicate the type of manure applied: S_l, liquid swine (*Sus scrofa domesticus*); P, poultry; BS, beef cattle (*Bos taurus*) and swine; D, dairy cattle.
‡‡ PPNT, preplant NO_3 test. ND, not determined.
§§ PSNT, presidedress NO_3 test.
¶¶ Grain yields are reported as a mean from four replicate check plots. Values in parentheses indicate N applied (kg ha^{-1}) prior to the growing season.
Calculated as 100 × (optimal yield − check-plot yield)/check-plot yield.

Table 2. Selected properties of additional soils used in developing soil N test.

Soil No.	Series	Subgroup	pH†	Organic C	Total N	Sand	Silt	Clay
				g kg^{-1}				
26	Elliott	fine, illitic, mesic Aquic Argiudoll	5.8	19.2	1.67	103	574	323
27	Drummer	fine-silty, mixed, superactive, mesic Typic Endoaquoll	6.0	31.5	2.53	170	480	350
28	Harpster	fine-silty, mesic Typic Calciaquoll	7.5	39.3	3.88	100	530	370
29	Houghton	euic, mesic Typic Haplosaprist	6.9	80.6	6.34	ND‡	ND	ND

† Soil/water ratio, 1:1.
‡ ND, not determined.

obtained when a thermometer was immersed in 100 mL of deionized water in a Mason jar placed in the center of the griddle.

Microburette or automatic titrator. Titrations were performed using a 5-mL microburette or a Metrohm Model 678 EP/KF Processor equipped with a Model 665 Dosimat (Metrohm, Herisau, Switzerland) and a combination electrode designed for flat-surface measurements (Model 13-620-289; Fisher Scientific, Pittsburgh, PA).

Reagents

Sodium hydroxide solution (2 *M*). Reagent-grade NaOH pellets (80 g) were dissolved in ~800 mL of deionized water in a 1-L volumetric flask. After cooling, the solution was diluted to 1 L and mixed thoroughly. The flask was kept tightly stoppered to minimize absorption of atmospheric CO_2 during storage of the NaOH solution. Alternatively, 2 *M* NaOH is available from Fisher Scientific (cat. no. LC24380).

Boric acid-indicator solution. A reagent containing 40 g of H_3BO_3 L^{-1} was prepared as described by Mulvaney (1996), Khan et al. (1997), and Mulvaney et al. (1997a). Alternatively, a suitable reagent may be obtained from Fisher Scientific (cat. no. LC11750).

Dilute sulfuric acid (0.01 *M* standard). This reagent was prepared by adding 5.6 mL of concentrated (18 *M*) H_2SO_4 to 10 L of deionized water in a 10-L Pyrex solution bottle (Corning Glass Works, Corning, NY). After thorough mixing with a motorized stirrer, the solution was standardized by titrating several 5-mL aliquots of a THAM solution that was prepared by dissolving 0.2430 g of dried, certified THAM (Sigma, St. Louis, MO) in 100 mL of deionized water in a volumetric flask. The endpoint for these titrations was determined as described in the procedure that follows. The molarity of the titrant was calculated as $0.05/V$, where V is the mean value for the milliliters of H_2SO_4 required to reach the endpoint. The calculated molarity was multiplied by 28 000 to obtain the titer (μg N mL^{-1}). Alternatively, standard 0.01 *M* (0.02 *N*) H_2SO_4 may be purchased from Fisher Scientific (cat. no. SA226).

Procedure

A 1-g sample of air-dried soil (<2 mm) was weighed into a Mason jar. A petri dish was attached to the jar lid with a cable tie, and 5 mL of H_3BO_3-indicator solution was dispensed into the dish. The soil sample was then treated with 10 mL of 2 *M* NaOH, and the jar was swirled to mix the contents, while taking care to minimize soil adherence to the wall of the jar. Within 15 to 30 s, the lid was placed on the jar and sealed with a screw band, and the jar was transferred to the hot plate. After 5 h, the jar was removed from the hot plate and opened, and the petri dish was released from the jar lid. The H_3BO_3 solution was diluted with 5 mL of deionized water, and subsequently titrated with 0.01 *M* H_2SO_4. Prior to titration, 5 mL of H_3BO_3 solution was dispensed into a petri dish containing 5 mL of deionized water, and the endpoint was established on the basis of the resulting color (for manual titrations) or pH (for automatic titrations). The micrograms of N liberated by diffusion was calculated as $S \times T$, where S is the volume of H_2SO_4 used in titration of the sample and T is the titer of the titrant (for 0.01 *M* H_2SO_4, $T = 280$ μg N mL^{-1}).

Development of Soil Test Method

The addition of NaOH and the diffusion period specified in the method described were established through comparative studies involving treatment of soil samples (four replicates) with 10 mL of 1, 2, or 5 *M* NaOH, followed by heating at 48 to 50°C for 1, 2, 3, 4, 5, 6, 7, 8, 12, or 24 h. The soils selected for use in these studies included the responsive and nonresponsive soils that had the highest and lowest concentrations of amino sugar N.

A study was conducted to ascertain whether any recovery of nonexchangeable NH_4-N occurs by the method described, by comparing soil test values obtained with KOH versus NaOH. The soils used in this study were selected on the basis of nonexchangeable NH_4-N concentration determined as described by Mulvaney (1996), and included two nonresponsive soils, a responsive soil, and three of the soils listed in Table 2. In each case, analyses were performed (four replicates) as specified, or by substituting 2 *M* KOH for 2 *M* NaOH.

Recovery tests were performed using ^{15}N to ensure that the method described estimates (NH_4 + amino sugar)-N but not NO_3-N, NO_2-N, or amino acid N. Four soils were used in this study, including one from a manure disposal site. Analyses were performed (four replicates) as specified to serve as a control, or after addition of 1 mL of deionized water containing 300 μg of N as $(NH_4)_2SO_4$ (1.422 atom % ^{15}N), glucosamine HCl (1.386 atom % ^{15}N), KNO_3 (1.646 atom % ^{15}N), $NaNO_2$ (1.534 atom % ^{15}N), or glycine (1.142 atom % ^{15}N). The labeled N was added following NaOH to prevent NH_4 fixation, and the jar was then sealed immediately to avoid gaseous loss of NH_3. Following quantitative determinations by titration, isotope-ratio analyses were performed on each sample by the Rittenberg process, using an automated system described in detail by Mulvaney et al. (1990), Mulvaney and Liu (1991), and Mulvaney et al. (1997b). Percentage recovery (R) was calculated as

$$R = M(T - U)/3(L - U) \quad [1]$$

where M is the micrograms of N collected during analysis of the treated sample, and the remaining variables represent measured (uncorrected) values of atom % ^{15}N for the treated sample (T), the untreated sample (U), and the labeled N added (L).

In a study to evaluate the effect of sampling depth on soil test values, analyses were performed (four replicates) by the method described on profile samples of two responsive and two nonresponsive soils, representing depths of 0 to 15, 15 to 30, and 30 to 60 cm.

To elucidate the importance of proper temperature control during the 5-h diffusion period specified, analyses were carried out with heating at 40, 44, 46, 48, 50, 52, 56, or 60°C. In each case, there were four replicate samples of two nonresponsive and two responsive soils. The soils used were selected so that both groups would vary widely in organic C.

The importance of aggregate size in the soil test described was investigated by performing analyses on samples of soil

that had been crushed to pass through a 2.0-mm screen, with or without further crushing to <0.15 mm. This investigation was carried out using the four soils listed in Table 2, and involved four replicate determinations.

Data Analysis

Data from replicate determinations were characterized by computing means and standard deviations. In some cases, mean values were compared on the basis of a least significant difference (LSD) at the 0.001 probability level, or by performing pairwise *t*-tests. Simple correlation or regression analyses were employed to quantify the linear relationship between soil test N and hydrolyzable amino sugar N, using data obtained for the 25 soils listed in Table 1. The ability of the soil test described to differentiate nonresponsive from responsive sites was evaluated by plotting the fertilizer response data for these sites versus their soil test values.

RESULTS AND DISCUSSION

Corn is seldom grown commercially without N fertilization, although a profitable yield response is not always obtained in high-input agriculture. This was the case with 12 of the 25 soils listed in Table 1, despite the fact that none of these sites was subject to drought, and most exceeded the soil test goals for cash-grain cropping in Illinois with regard to pH (6.0), Bray-1 P (≥45–56 kg ha^{-1}), and exchangeable K (≥290–335 kg ha^{-1}). Of this group, eight soils had been manured, but the rate of manure N application varied from 85 to >2500 kg ha^{-1} and far exceeded crop N requirements at three sites that had been used for manure disposal (Soils 1, 2, and 5). Corn followed alfalfa (*Medicago sativa* L.) at one of the remaining nonresponsive sites (Soil 3), while no-till soybean was the previous crop at another (Soil 12). Soils 8 and 11 were collected from unmanured sites under continuous corn, but a high level of Bray-1 P and/or exchangeable K suggested the possibility of previous manuring or excessive fertilization.

On the basis of the proven-yield approach described in the Illinois Agronomy Handbook (1998) to guide N-fertilizer recommendations for corn, six of the 12 nonresponsive sites listed in Table 1 would have been identified correctly for their complete lack of yield response to N fertilization. In each case, the N credit from manure (190 kg manure N ha^{-1}) would have exceeded the N requirement estimated for the yield goal, although this sometimes occurred only because of an additional N credit for corn following soybean (48 kg ha^{-1}). None of the remaining six sites in this group would have been identified by the proven-yield approach as being nonresponsive to N fertilization, and therefore would have been fertilized at an economic loss to the farmer and with the risk of causing environmental pollution. Overfertilization would have been more extensive for Soils 8 and 11, which came from sites where continuous corn was grown without manure, than for Soils 3, 6, 10, and 12, in which case the use of manure or a previous legume led to an N credit.

Soil testing for NO_3 was done by Brown et al. (1993) to evaluate the PPNT and PSNT for identifying sites that do not require N fertilization for corn production, as a possible improvement over the proven-yield approach. The data thereby obtained for the 25 soils used in our work are included in Table 1. Assuming a critical value of 16 mg N kg^{-1} (Schmitt and Randall, 1994), four of the 12 nonresponsive sites were detected by the PPNT. The PSNT was somewhat more effective in identifying six of these sites, relative to a critical value of 21 mg N kg^{-1} (Fox et al., 1989; Bundy and Andraski, 1993), but provided no improvement over the proven-yield approach, which also identified six of the 12 nonresponsive sites. Most of the soils having a high concentration of NO_3 had been manured, although several such sites were not identified by either the PPNT or the PSNT, including one used to dispose of liquid swine (*Sus scrofa domesticus*) manure (Soil 2). A nonresponsive site where first-year corn followed alfalfa (Soil 3) was identified by the PSNT, but not by the PPNT.

Recent work in our laboratory suggests that a soil test could be developed to estimate the supply of plant-available N, based on chemical analyses for a specific fraction of soil organic N that is highly mineralizable. This fraction was identified by comparing N-distribution analyses for soils that differed in whether N fertilization had effected a yield response by corn, using diffusion methods developed by Mulvaney and Khan (2001). The results showed 11 nonresponsive soils to be significantly higher than seven responsive soils in their concentrations of amino sugar N, whereas no consistent difference was observed for total hydrolyzable N, hydrolyzable NH_4-N, or amino acid N (Mulvaney et al., 2001). The same finding applies in the present study, which involved a larger number of soils representing additional nonresponsive and responsive sites. This is apparent from Table 3, in that the two groups were completely resolved ($P < 0.001$) on the basis of amino sugar N. The lowest value for any nonresponsive soil was 34% higher than the highest value for any responsive soil, and on average, the difference in amino sugar N was more than 200%.

Although Table 3 demonstrates that hydrolyzable amino sugar N can be determined to identify sites where corn does not respond to N fertilization, the techniques involved are too laborious and time-consuming for use in routine soil testing. These limitations arise largely from the need to prepare a neutralized soil hydrolysate, whereas the analysis to determine hydrolyzable (NH_4 + amino sugar)-N is readily accomplished by carrying out diffusions with strong alkali, which effects chemical deamination of free amino sugars (Mulvaney and Khan, 2001). Based on previous work to develop direct-diffusion methods for inorganic N analysis of soil (Khan et al., 2000), a soil test was developed to estimate amino sugar N, in which alkalization is performed directly on the soil itself, rather than on a soil hydrolysate.

To optimize reaction conditions for liberating amino sugar N from soil, studies were conducted to compare different concentrations of NaOH and different diffusion periods, in which diffusions were performed at 48 to 50°C on a hot plate to promote the alkaline decomposition of amino sugars (Mulvaney and Khan, 2001). The results (Fig. 1) were obtained using the two responsive

Table 3. Concentrations of hydrolyzable N in soil from N-response study sites.†

	Form of hydrolyzable N							
	Total		Amino acid		NH_4		Amino sugar‡	
Soil no.	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	mg N kg^{-1}							
	Nonresponsive sites							
1	1928	13.4	695	3.8	482	8.6	412	16.0
2	1246	11.5	419	1.1	193	2.2	411	7.8
3	1634	24.0	537	6.8	333	3.0	267	12.5
4	2227	10.5	757	2.0	499	33.0	502	25.6
5	3022	27.2	908	11.4	604	13.3	511	18.5
6	1866	28.8	536	3.6	435	12.5	260	12.8
7	1396	5.4	470	4.3	369	16.8	350	17.9
8	996	7.1	340	5.3	252	6.6	323	12.8
9	1805	15.3	1180	6.2	304	0.7	343	2.4
10	1792	7.0	764	5.6	516	13.7	314	28.9
11	1412	6.0	520	11.5	266	9.0	360	5.0
12	1673	6.0	649	4.2	439	11.0	303	14.7
	Responsive sites							
13	1280	3.6	346	4.9	435	6.0	116	8.1
14	905	6.3	214	1.7	229	1.9	119	4.6
15	1509	2.3	537	3.4	447	3.5	192	11.3
16	857	14.3	350	2.6	260	12.5	80	4.7
17	935	3.8	375	7.6	316	12.1	129	2.0
18	643	4.1	280	1.4	181	4.4	83	2.5
19	1030	3.8	379	2.0	296	3.7	139	13.9
20	1541	5.2	531	6.7	354	19.4	194	12.8
21	1134	8.1	279	2.4	293	2.9	139	5.2
22	1506	5.5	459	5.1	413	1.7	74	4.3
23	1145	4.7	394	1.7	380	2.6	124	11.3
24	620	6.2	177	7.1	192	1.1	67	2.5
25	593	19.2	70	9.1	182	6.7	46	3.2
LSD (0.001)	30		14		27		31	

† Determinations were performed on four replicate samples of soil. SD, standard deviation for replicate determinations.
‡ Determined as (NH_4 + amino sugar)-N–NH_4-N.

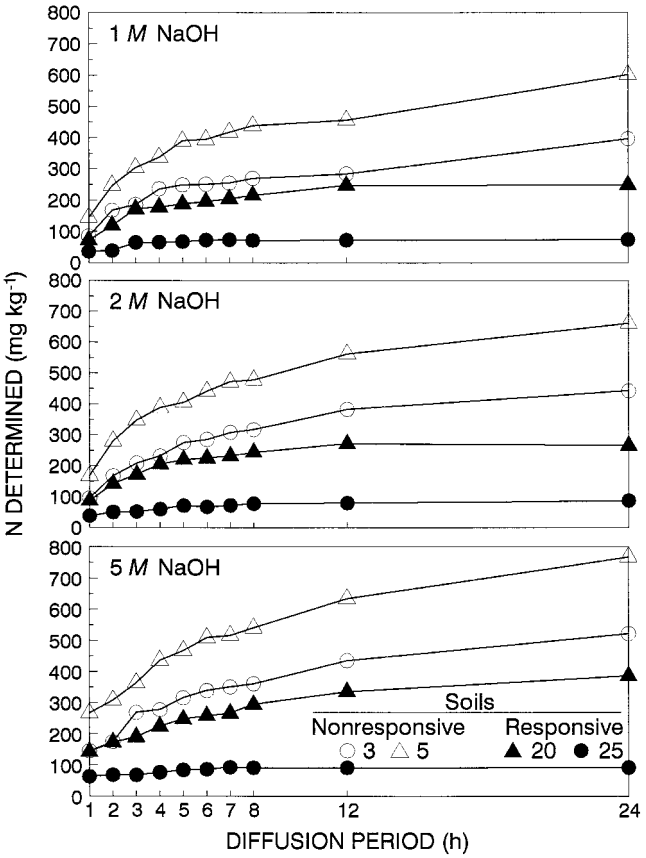

Fig. 1. Effectiveness of different additions of NaOH for determination of potentially available soil N during diffusion (four replicates) at 48 to 50°C. The standard deviation for replicate determinations did not exceed 5.1 mg N kg^{-1}.

and the two nonresponsive soils that had the highest and lowest concentrations of amino sugar N. As expected, a larger amount of N was liberated when diffusion was performed with a higher concentration of NaOH or for a longer period. A 2 *M* reagent and a 5-h diffusion period were adopted in the soil test described as the best compromise in terms of speed, convenience, sensitivity, and resolution. A longer diffusion period was required with 1 *M* NaOH to clearly differentiate responsive from nonresponsive soils, whereas the diffusion period was much more critical with 5 *M* NaOH because of less temporal stability in soil test values. With 2 *M* NaOH, a 5-h diffusion period was adequate to easily resolve the two nonresponsive soils from the two responsive soils, and even provided sufficient resolution to clearly distinguish among all four of these soils. Moreover, the data in Fig. 1 show that soil test values obtained with 2 *M* NaOH do not increase appreciably if diffusion is continued beyond the 5-h period specified, although this practice should be avoided, since prolonged heating may lead to drying of the H_3BO_3 solution used to absorb gaseous NH_3 and thereby vitiate the analysis. The latter problem does not occur if heating is discontinued after 5 h, as very little, if any, change has been observed in soil test values by leaving the jar unopened overnight at room temperature (25°C). This could be a valuable option in processing a large number of soil samples, as is often necessary in soil testing laboratories.

Besides amino sugar N, the soil test described recovers any exchangeable NH_4-N that may be present in the sample under analysis. This was not a concern in our work, as analyses by direct diffusion (Khan et al., 2000) showed that the soils listed in Table 1 contained very little exchangeable NH_4 (10–17 mg N kg^{-1} for nonresponsive soils, 10–15 mg N kg^{-1} for responsive soils). Some of these soils, however, contained a substantial amount of nonexchangeable NH_4, which would increase soil test values if liberated by the method described. To check this possibility, a study was done to ascertain whether soil test values are affected by substituting KOH for NaOH, using seven soils that ranged widely in their content of nonexchangeable NH_4. The results (Table 4) show very little difference between the two alkaline reagents, whereas higher values would have been obtained with NaOH if the soil test method caused substantial release of nonexchangeable NH_4-N, owing to the blocking effect of K on this release.

Further evidence that the soil test described estimates (NH_4 + amino sugar)-N is provided by Table 5, which shows the results of ^{15}N recovery tests in which soil samples were treated with labeled $(NH_4)_2SO_4$, glucosamine, KNO_3, $NaNO_2$, or glycine. As expected, recovery of ^{15}N was quantitative with NH_4 or glucosamine and undetectable with NO_3 or NO_2. Recovery with labeled glycine ranged from 4 to 6.5%, which was somewhat higher than had been observed in previous ^{15}N recovery

Table 4. Comparison of NaOH and KOH for liberation of alkali-labile soil N.†

Soil no.	Nonexchangeable NH_4	NaOH‡		KOH‡		Difference§
		Mean	SD	Mean	SD	
	mg N kg^{-1}					%
3	200	276	2.6	271	5.6	−1.7
5	181	435	2.2	431	4.8	−0.9
20	70	221	1.5	215	6.7	−2.4
25	104	72	2.0	69	0.9	−4.7
26	158	200	1.2	206	1.5	2.6
27	134	298	5.2	288	6.6	−3.2
29	138	671	1.3	669	2.6	−0.4

† Diffusions were performed for 5 h at 48 to 50°C after treatment of soil (1 g) with 10 mL of 2 *M* NaOH or KOH.
‡ Four determinations. SD, standard deviation.
§ Calculated as 100 × (N_{KOH} − N_{NaOH})/N_{NaOH}.

tests with soil hydrolysates (Mulvaney and Khan, 2001). The latter finding is probably of no concern given the limited magnitude of recovery, particularly since the amino acids in soil occur almost exclusively in combination with humic colloids or clay minerals (Stevenson, 1994), and would be much more stable than a purified amino acid added in solution.

Because diffusions with NaOH recover exchangeable NH_4-N as well as amino sugar N, the procedure described will not provide a reliable estimate of mineralizable soil N for sites that have received a recent input of NH_4 through application of ammoniacal fertilizer, manure, or sewage sludge. The need for this sort of information will normally not exist in such cases, but if necessary, amino sugar N can be estimated by correcting soil test values on the basis of NH_4-N analyses by direct diffusion (Khan et al., 2000).

The soil test described could have been easily modified to recover (NO_3 + NO_2)-N through addition of Devarda's alloy as a reducing agent, but this modification was omitted deliberately, so as to avoid two problems that result from the dynamic nature and mobility of soil NO_3. One of these problems is the extensive spatial and temporal variability that occurs in soil NO_3 concentrations (Lockman and Storer, 1990; Cahn et al., 1994; Hergert et al., 1995; Everett and Pierce, 1996), which would reduce soil test reliability in detecting sites that do not need N fertilization. The other is the need for profile sampling to account for downward movement of NO_3 through leaching. Such sampling is incompatible with routine soil testing for pH, P, and K, which normally involves sampling to a depth of 15 to 18 cm. The soil samples used in our work were collected from the 30-cm surface; however, a study to compare N-test values for different profile depths (Table 6) showed that the highest values were obtained for the 15-cm surface, and that a decrease occurred with greater depth. This is exactly what would be expected for an organic fraction of soil N that is subject to little, if any, transport by leaching. Table 6 suggests that standard sampling techniques would be appropriate for the soil test described, and that testing could be done in conjunction with normal soil tests for pH, P, and K. Table 6 also indicates that testing could be done with any tillage system, since N-test values for the four soils used followed the same order, regardless of sampling depth.

Table 5. Recovery by soil test method described of ^{15}N added to soil samples.†

Soil no.	Form of ^{15}N added‡									
	NH_4		Glucosamine		NO_3		NO_2		Glycine	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	% recovery									
	Nonresponsive sites									
5	98.8	0.4	99.7	0.6	ND	–	ND	–	6.5	0.4
8	102.6	2.0	101.3	1.8	ND	–	ND	–	4.3	0.8
	Responsive sites									
14	101.8	1.8	98.6	1.4	ND	–	ND	–	4.7	0.3
20	98.0	3.2	97.2	2.1	ND	–	ND	–	4.5	0.3

† Analyses were performed on soil samples (1 g) that had been treated with 0 or 300 μg of N as $(NH_4)_2SO_4$ (1.422 atom % ^{15}N), glucosamine HCl (1.386 atom % ^{15}N), KNO_3, (1.646 atom % ^{15}N), $NaNO_2$ (1.534 atom % ^{15}N), or glycine (1.142 atom % ^{15}N).
‡ Percentage recovery was calculated by Eq. [1]. Four replicates. SD, standard deviation. ND, not detected.

Heating is essential to promote the alkaline decomposition of amino sugars (Mulvaney and Khan, 2001), so a hot plate is used in the soil test described to carry out diffusions after treating the sample with NaOH. Figure 2 shows the results from a study to compare soil test values when diffusions were done for 5 h at various temperatures ranging from 40 to 60°C, so as to evaluate the need for proper temperature control in the method described. The data in Fig. 2 demonstrate quite clearly the potential for error if diffusions are not performed between 48 and 50°C as specified. Below 48°C, a 5-h diffusion period was inadequate for reliable resolution of a nonresponsive soil (Soil 8) and a responsive soil (Soil 20) that differed by <5% in their concentrations of hydrolyzable (NH_4 + amino sugar)-N (Table 3). Above 50°C, more extensive liberation of amino sugar N should have led to higher resolution in differentiating responsive and nonresponsive soils, but resolution was actually sacrificed when heating was done at 52 to 60°C. This decline was due in part to a reduction in the capacity of H_3BO_3-indicator solution for absorption of gaseous NH_3 (Khan et al., 1997), which accounts for the decrease

Table 6. Effect of profile depth on soil N-test values.†

Soil no.	Sampling depth	N determined‡	
		Mean	SD
	cm	mg kg^{-1}	
	Nonresponsive sites		
9	0–15	404	1.0
	15–30	300	1.4
	30–60	148	4.1
10	0–15	274	1.6
	15–30	207	3.3
	30–60	112	2.1
	Responsive sites		
14	0–15	192	1.4
	15–30	152	3.0
	30–60	86	0.9
18	0–15	128	1.2
	15–30	71	2.4
	30–60	69	0.5

† Diffusions were performed for 5 h at 48 to 50°C after treatment of soil (1 g) with 10 mL of 2 *M* NaOH.
‡ Four determinations. SD, standard deviation.

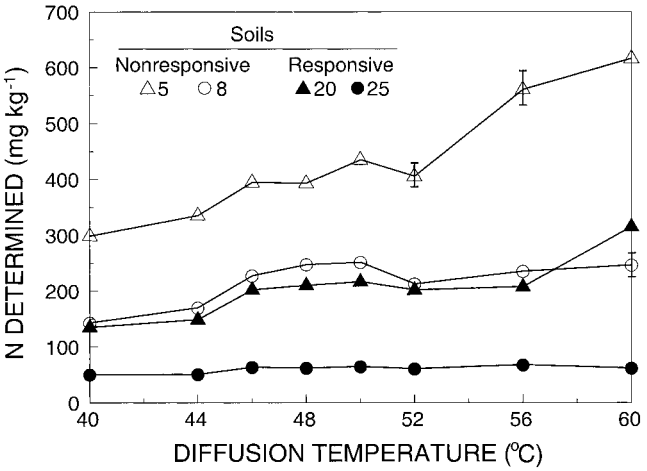

Fig. 2. Effect of temperature on determination of potentially available soil N by diffusion method described. Diffusions were performed (four replicates) for 5 h at 40 to 60°C, after treatment of soil (1 g) with 10 mL of 2 *M* NaOH. Error bars representing one standard deviation above and below the mean are shown when they exceeded the size of the data marker.

observed in soil test values when the temperature was raised from 50 to 52°C (Fig. 2). Heating at 56 or 60°C led to higher N-test values for a responsive and a nonresponsive soil, whereas very little change occurred with the other two soils. The latter finding can probably be attributed to the decomposition of soil organic matter constituents besides amino sugars, since soil concentrations of organic C and total N were much greater in the two cases where overheating led to an increase in N-test value (Table 1). The effect in one of these cases was a higher N-test value for a responsive soil (Soil 20) than for a nonresponsive soil (Soil 8), which could lead to the erroneous conclusion that a responsive soil does not require N fertilization.

Besides demonstrating the need to avoid under or overheating in the soil test described, Fig. 2 suggests that proper temperature control is crucial for reliable estimation of potentially available soil N. Unfortunately, this factor appears to have been largely ignored in previous work to develop chemical indices of soil N availability based on alkaline hydrolysis, which have involved either diffusion at room temperature (e.g., Cornfield, 1960; Chu, 1962; Keeney and Bremner, 1966; Cornforth and Walmsley, 1971; Walmsley and Forde, 1976) or steam distillation (e.g., Prasad, 1965; Jenkinson, 1968; Stanford and Legg, 1968; Cornforth and Walmsley, 1971). Without heating, alkaline hydrolysis is ineffective for liberating amino sugar N (Mulvaney and Khan, 2001), whereas determinations at 100°C would include other forms of soil organic N that may not be mineralized readily. The latter problem likewise precludes the possibility that soil N availability can be estimated accurately on the basis of organic matter or total N content.

Table 7. Effect of soil aggregate size on N-test values.†

	Aggregate size (mm)‡			
	<2		<0.15	
Soil no.	Mean	SD	Mean	SD
	mg kg^{-1}			
26	200	1.2	202	1.1
27	300	5.2	302	1.0
28	357	1.4	359	1.1
29	671	2.0	668	2.5

† Diffusions were performed for 5 h at 48 to 50°C after treatment of soil (1 g) with 10 mL of 2 *M* NaOH.
‡ Four determinations. SD, standard deviation.

Table 8. N-test values for soils differing in N-fertilizer responsiveness.†

	N determined‡	
Soil no.	Mean	SD
	mg kg^{-1}	
	Nonresponsive sites	
1	305	1.5
2	247	4.0
3	276	2.6
4	380	2.6
5	435	2.2
6	285	0.3
7	275	4.9
8	248	0.2
9	302	0.9
10	366	2.8
11	237	2.5
12	305	3.5
	Responsive sites	
13	172	3.2
14	158	2.3
15	223	2.3
16	115	1.8
17	169	2.3
18	117	1.4
19	187	2.3
20	221	1.5
21	190	2.1
22	159	4.2
23	184	3.0
24	153	1.6
25	72	2.0
LSD (0.001)	6	

† Diffusions were performed for 5 h at 48 to 50°C after treatment of surface (0–30 cm) soil (1 g) with 10 mL of 2 *M* NaOH.
‡ Four determinations. SD, standard deviation.

The soil samples used in the present project were ground much more finely than samples for routine soil testing, which are usually screened to <2 mm to remove organic detritus or inert rock fragments. A study to compare soil test values for samples differing in aggregate size (Table 7) showed no appreciable difference with and without fine grinding (to <0.15 mm), as would be expected from the fact that soil aggregates quickly form a slurry when treated with 10 mL of 2 *M* NaOH. This suggests that the soil test described will require no special processing of soil samples.

The validity of the soil test described was evaluated using soil samples from all 25 of the sites listed in Table 1, 12 of which had been identified correctly as being nonresponsive to N fertilization on the basis of hydrolyzable amino sugar N. The results (Table 8) show that, as with hydrolyzable amino sugar N, higher values were obtained for all 12 of the nonresponsive soils than for any of the 13 responsive soils. The difference between these groups was smaller than with hydrolyzable amino sugar N (Table 3), but analytical precision benefited considerably from the fact that titration data obtained by the soil test method indicate directly the milligrams

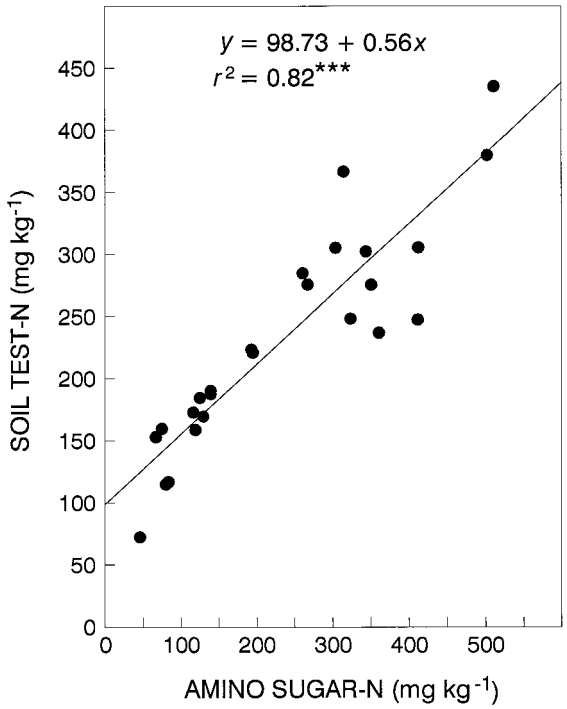

Fig. 3. Relationship between soil test N and hydrolyzable amino sugar N for surface (0–30 cm) samples of 25 Illinois soils identified in Table 1. Both parameters were determined as the mean of four replicate analyses.

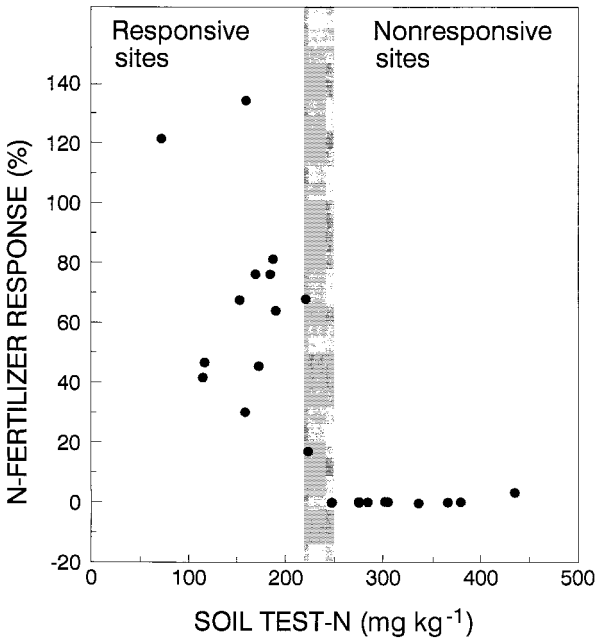

Fig. 4. Relationship between soil test N and N fertilizer response for soils from 25 N-response sites listed in Table 1. Values for N-fertilizer response were calculated as 100 × (optimum yield – check-plot yield)/check-plot yield, using yield data reported by Brown (1996). Values for soil test N were obtained as the mean of four determinations.

of N per kilogram, and complete resolution was maintained at the 0.001 level. Comparison of Tables 3 and 8 reveals that soil test values tended to follow the same order as analyses for hydrolyzable amino sugar N. The close quantitative relationship between these parameters is confirmed by Fig. 3, which shows that they were correlated very highly significantly ($r = 0.90$***).

The potential value of the soil test described for detecting sites where corn does not respond to fertilizer N is evident from Fig. 4, in which the percentage yield response to N fertilization is plotted versus soil test-N for the 12 nonresponsive and the 13 responsive soils listed in Table 1. A critical range of 225 to 235 mg N kg^{-1} was appropriate to completely resolve the two groups, although this range will necessarily depend upon sampling depth, based on the data in Table 6.

Further examination of Fig. 4 reveals a considerable range in N-test values for either nonresponsive or responsive soils, which suggests the possibility of a quantitative soil test as well as a means of detecting nonresponsive sites. One application for such a test would be to estimate how long a soil will remain nonresponsive. Another would be to serve as the basis for N-fertilizer applications to responsive soils, perhaps in conjunction with climatic data and a soil productivity index.

SUMMARY AND CONCLUSIONS

A soil test was developed to estimate (NH_4 + amino sugar)-N, as a much more convenient alternative to N-distribution analysis of soil hydrolysates. Besides providing an unprecedented capability to detect sites where there is no yield response by corn to N fertilization, the soil test described is unsurpassed in simplicity and convenience. No specialized or expensive equipment is required, and the reagents are nonhazardous and can be purchased commercially. The test procedure is performed directly on the soil sample, without the need for extraction, as is required in conventional soil tests for available P and K. By design, NO_3-N is not recovered, so as to reduce soil test variability and eliminate the need for profile sampling and special care in sample processing. On the basis of soil test N, 25 Illinois soils (0–30 cm) were classified correctly as being responsive (<225 mg kg^{-1}) or nonresponsive (>235 mg kg^{-1}) to N fertilization.

The soil test described has obvious value for improving N-fertilizer efficiency, increasing the profitability of corn production, and reducing the adverse environmental effects of excessive N fertilization.

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